

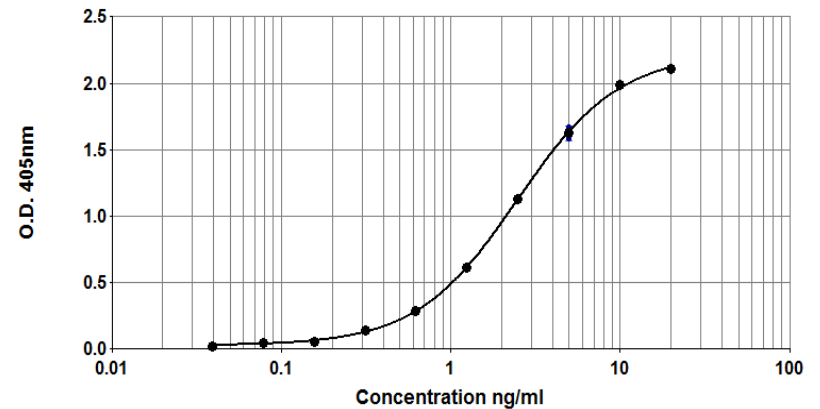


Equ c 4 ELISA kit (103/14G4)

Product Code: EL-EC4

Lot Number: xxxxx

Sample Curve:



Content:

- Vial 1 (red top) 100 μ L
Monoclonal antibody 103
Concentration: 1mg/ml in PBS
- Vial 2 (white top) 400 μ L
Equ c 4 Standard
Concentration: 200U/ml Equ c 4
- Vial 3 (brown) 100 μ L
Biotinylated monoclonal antibody 14G4
Dilute: 1:1000 for use

Storage: The ELISA kit should be stored at 4°C

For research and commercial use in vitro: not for human in vivo or therapeutic use.

Certificate of Analysis

Monoclonal Antibody: 103
Immunogen: Horse Allergen
Specificity: Binds to an epitope on horse *Equus caballus* allergen, Equ c 4.
Concentration: 1.0mg/ml in phosphate buffered saline. Based on A280 for IgG (1.42=1mg/ml) 0.22µm filtered, 0.02% sodium azide.
Lot Number: xxxxx

Monoclonal Antibody: 14G4
Immunogen: Horse Allergen
Specificity: Binds to an epitope on horse *Equus caballus* allergen, Equ c 4.
Concentration: 1.0mg/ml in phosphate buffered saline. Based on A280 for IgG (1.42=1mg/ml) 0.22µm filtered, 0.02% sodium azide.
Biotinylation: Biotinylated and titrated for use in ELISA at 1/1000 dilution.
Lot Number: xxxxx

Allergen Standard: Equ c 4 Standard
Composition: Horse hair extract prepared in 1% BSA/50% glycerol/PBS, pH 7.4
Concentration: 200U/ml
Lot Number xxxxx

ELISA Protocol for Equ c 4.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 103 at 10µl/10ml, i.e. 1/1000 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, incubate overnight at 4°C.
2. Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100µl/well of 1% BSA, PBS-T. Wash 3x with PBS-T.
3. Use doubling dilutions of the Horse Standard to make a control curve ranging from 20 - 0.04U/ml Equ c 4: Pipette 20µl standard into 180µl 1% BSA, PBS-T into wells A1 and B1 on the ELISA plate. Mix well and transfer 100µl across the plate into 100µl 1% BSA, PBS-T diluent to make 10 serial doubling dilutions. Wells A11, B11 and A12, B12 should contain only 1% BSA, PBS-T as blanks.
4. Add 100µl of diluted allergen samples and incubate for 1 hour at room temperature. House dust extracts for Equ c 4 analysis are routinely diluted two-fold from 1/10-1/80. Other sample types, like air filter extracts and allergen extracts, may require different dilutions.
5. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Equ c 4 mAb 14G4. The antibody solution should be diluted 1/1000 in 1% BSA, PBS-T. Incubate for 1 hour at room temperature.
6. Wash wells 3x with PBS-T and add 100µl diluted Streptavidin-Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water). The reconstituted Streptavidin should be diluted 1/1000 in 1% BSA, PBS-T. Incubate for 30 minutes at room temperature.
7. Wash wells 3x and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H₂O₂. Read the plate when the absorbance at 405nm reaches 2.0-2.4.

Notes:

The Horse Allergen Standard is recommended for immunoassay calibration purposes only. Not recommended for in-vitro antibody measurements, T cell studies, immunization purposes, or other uses.

Buffer recipes, storage conditions and a list of frequently asked questions can be found under "Protocols" on our web site: www.inbio.com.

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